

What is claimed is:

1. A process for detecting the presence or absence of methylation of a CpG dinucleotide rich region of a nucleic acid sequence within a genome, the process comprising:
  - 5 a. contacting the nucleic acid sequence with an enzyme which digests the nucleic acid sequences into fragments in which CpG islands are preserved;
  - b. attaching the fragments to linker primers to form linker primer products;
  - 10 c. contacting the linker primer products with a methylation-sensitive enzyme which digests the linker products having unmethylated CpG dinucleotide sequences but not methylated CpG dinucleotide sequences to form a digestion product comprising
  - 15 methylated CpG island loci;
  - d. amplifying the digestion product to form amplicons;
  - e. labeling the amplicons;
  - f. contacting the labeled amplicons with a screening array comprising a plurality of nucleic acid
  - 20 fragments affixed to a solid support; and
  - g. determining the presence or absence of labeled amplicons bound to the plurality of nucleic acid fragments affixed to the solid support of the screening array.

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2. The process of claim 1 wherein the plurality of nucleic acid fragments affixed to the solid support of the screening array are derived from a CpG dinucleotide rich genomic library.
3. The process of claim 2 wherein the nucleic acid fragments affixed to the solid support of the screening array are CpG dinucleotide rich fragments which comprise a sequence of at least about 200 nucleotides of which at least about  
5 50% are guanine and cytosine.
4. The process of claim 3 wherein at least 20 nucleic acid fragments are affixed to the solid support of the screening array.
5. The process of claim 3 wherein the plurality of nucleic acid fragments affixed to the solid support of the screening array each contain a promoter and a first exon of a gene.
6. The process of claim 5 wherein the plurality of nucleic acid fragments affixed to the solid support of the screening array each comprise a nucleic acid sequence which is expressed in an organism.
7. The process of claim 6<sup>1</sup> wherein at least 20 nucleic acid fragments are affixed to the solid support of the screening array.

8. The process of claim 7 wherein at least 100 nucleic acid fragments are affixed to the solid support of the screening array.
9. The process of claim 8 wherein at least 500 nucleic acid fragments are affixed to the solid support of the screening array.
10. The process of claim 1 wherein the solid support of the screening array comprises nylon, glass or silicon.
11. The process of claim 1 wherein the label is selected from the group consisting of radioisotopes and fluorescent labels.
12. The process of claim 1 wherein the enzyme is selected from the group consisting of MseI, Tsp509I, NlaIII and BfaI and the methylation sensitive enzyme is selected from the group consisting of BstU I, SmaI, SacII, EagI, MspI, HpaII, HhaI and BssHII.
13. The process of claim 12 wherein the enzyme is MseI and the methylation sensitive enzyme is BstU I.
14. The process of claim 1 wherein said process is used for diagnosing and monitoring the prognosis of a disease associated with aberrant DNA methylation.

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15. The process of claim 14 wherein said disease is breast cancer, prostate cancer, colon cancer, lung cancer, liver cancer and ovarian cancer.
16. The process of claim 15 wherein the disease is breast cancer.
17. The process of claim 16 wherein the CpG island fragments affixed on the solid support of the screening array are selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46.
18. A screening array comprising a solid support and a plurality of nucleic acid fragments affixed to the solid support wherein each nucleic acid fragment of the plurality of the nucleic acid fragments is a CpG



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26. The screening array of claim 18 wherein the plurality of nucleic acid fragments affixed on the solid support are selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46.
27. A process for generating a screening array containing expressed gene sequences, said process comprising the steps of:
- contacting a nucleic acid sequence with an enzyme which digests the nucleic acid sequence into fragments in which CpG islands are preserved;
  - amplifying the fragments to form a plurality of CpG island fragments;

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- 5      c.    screening the plurality of CpG island fragments with  
         a nucleic acid probe to identify CpG island  
         fragments which contain expressed sequences; and  
         d.    affixing the CpG island fragments which contain  
5           expressed sequences onto a solid support.
28.    The process of claim 27 wherein the nucleic acid sequence  
         is derived from a CpG dinucleotide rich genomic library.
29.    The process of claim 28 wherein the CpG island fragments  
         which contain expressed sequences are CpG dinucleotide  
         rich fragments which comprise a sequence of at least  
         about 200 nucleotides of which at least about 50% are  
5           guanine and cytosine.
30.    The process of claim 29 wherein at least 20 CpG island  
         fragments which contain expressed sequences are affixed  
         to the solid support.
31.    The process of claim 29 wherein the CpG island fragments  
         which contain expressed sequences each contain a promoter  
         and a first exon of a gene.
32.    The process of claim 31 wherein at least 20 CpG island  
         fragments which contain expressed sequences are affixed  
         to the solid support.

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33. The process of claim 32 wherein at least 100 CpG island fragments which contain expressed sequences are affixed to the solid support.
34. The process of claim 33 wherein at least 500 CpG island fragments which contain expressed sequences are affixed to the solid support.
35. The process of claim 27 wherein the enzyme is selected from the group consisting MseI, Tsp509I, NlaIII and BfaI.
36. The process of claim 35 wherein the enzyme is MseI.
37. The process of claim 27 wherein the solid support comprises nylon, glass or silicon.
38. The process of claim 27 wherein the nucleic acid probe is isolated from normal breast, colon, ovarian, lung and prostate tissue samples.
39. The process of claim 38 wherein the nucleic acid probe is a cDNA probe.
40. A set of amplicons, the set comprising amplified nucleic acid fragments, the nucleic acid fragments being the digestion product of a genomic nucleic acid sequence which has been digested with restriction enzymes which

digest the nucleic acid sequence into fragments in which methylated CpG islands are preserved.

41. The set of amplicons of claim 40 wherein a first restriction enzyme is selected from the group consisting of MseI, Tsp509I, NlaIII and BfaI and a second restriction enzyme is a methylation sensitive enzyme.
42. The set of amplicons of claim 41 wherein the first restriction enzyme is MseI.
43. The set of amplicons of claim 42 wherein the methylation sensitive enzyme is selected from the group consisting of BstU I, SmaI, SacII, EagI, MspI, HpaII, HhaI and BssHII.
44. The set of amplicons of claim 43 wherein the methylation sensitive enzyme is BstU I.
45. A process for isolating a set of amplicons used to identify DNA methylation patterns, said process comprising:
  - a. contacting nucleic acid sequences with an enzyme which digests the nucleic acid sequences into fragments in which CpG islands are preserved;
  - b. attaching the fragments to linker primers to form linker primer products;



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51. A process of identifying methylation patterns in DNA from a cell sample, said process comprising:
  - a. isolating a first set of amplicons comprising (i) contacting nucleic acid sequences derived from a tumor cell with an enzyme which digests the nucleic acid sequences into fragments in which CpG islands are preserved; (ii) attaching the fragments to linker primers to form linker primer products; (iii) contacting the linker primer products with a methylation-sensitive enzyme which digests the linker primer products having unmethylated CpG dinucleotide sequences but not methylated CpG dinucleotide sequences to form a digestion product comprising methylated CpG island loci; (iv) amplifying the digestion product to form amplicons; and (v) labeling the amplicons;
  - b. isolating a second set of amplicons comprising repeating (i) through (v) of step (a) wherein the nucleic acid sequences of (i) are nucleic acid sequences derived from a non-tumor cell;
  - c. contacting the first set of amplicons with a first screening array comprising a plurality of nucleic acid fragments affixed to a solid support and determining the presence or absence of labeled amplicons bound to the plurality of nucleic acid fragments of the first screening array;

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- 5           d.    contacting the second set of amplicons with a second  
            screening array comprising a plurality of nucleic  
            acid fragments affixed to a solid support wherein  
            said plurality of nucleic acid fragments of the  
            second screening array is identical to the plurality  
            of nucleic acid fragments of the first screening  
            array and determining the presence or absence of  
            labeled amplicons bound to the plurality of nucleic  
            acid fragments of the second screening array; and  
10           e.    observing whether the presence or absence of the  
                  first set of amplicons bound to the plurality of  
                  nucleic acid fragments of the first screening array  
                  is the same as the presence or absence of the second  
                  set of amplicons bound to the plurality of the  
15                   nucleic acid fragments of the second screening  
                  array.
52.   The process of claim 51 wherein the tumor cell is a  
      breast cancer cell, prostate cancer cell, colon cancer  
      cell, lung cancer cell, liver cancer cell and ovarian  
      cancer cell.
53.   The process of claim 51 wherein the enzyme is selected  
      from the group comprising MseI, Tsp509I, NlaIII and BfaI  
      and the methylation sensitive enzyme is selected from the  
      group comprising BstU I, SmaI, SacII, EagI, MspI, HpaII,  
5       HhaI and BssHII.

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54. The process of claim 53 wherein the enzyme is MseI and the methylation sensitive enzyme is BstU I.
55. The process of claim 51 wherein the nucleic acid fragments affixed to the solid support of the first screening array and the plurality of nucleic acid fragments affixed to the solid support of the second  
5 screening array are derived from a CpG dinucleotide rich genomic library.
56. The process of claim 55 wherein the nucleic acid fragments affixed to the solid support of the first screening array and the plurality of nucleic acid fragments affixed to the solid support of the second  
5 screening array are CpG dinucleotide rich fragments which comprise a sequence of at least about 200 nucleotides of which at least about 50% are guanine and cytosine.
57. The process of claim 56 wherein at least 20 nucleic acid fragments are affixed to the solid support of the first screening array and the plurality of nucleic acid fragments affixed to the solid support of the second  
5 screening array.
58. The process of claim 56 wherein the plurality of nucleic acid fragments of the first screening array and the second screening array each contain a promoter and a first exon of a gene.

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59. The process of claim 58 wherein at least 20 nucleic acid fragments are affixed to the solid support of the first screening array and the plurality of nucleic acid fragments affixed to the solid support of the second screening array.
60. The process of claim 59 wherein at least 100 nucleic acid fragments are affixed to the solid support of the first screening array and the plurality of nucleic acid fragments affixed to the solid support of the second screening array.
61. The process of claim 60 wherein at least 500 nucleic acid fragments are affixed to the solid support of the first screening array and the plurality of nucleic acid fragments affixed to the solid support of the second screening array.
62. The process of claim 51 wherein the solid support of the first screening array and the solid support of the second screening array comprises nylon, glass or silicon.
63. The process of claim 51 wherein said process is used for diagnosing and monitoring the prognosis of disease associated with aberrant DNA methylation.

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64. The process of claim 63 wherein said disease is breast cancer, prostate cancer, colon cancer, lung cancer, liver cancer and ovarian cancer.

65. The process of claim 64 wherein the disease is breast cancer.

66. The process of claim 51 wherein the plurality of nucleic acid fragments affixed to the solid support of the first screening array and the plurality of nucleic acid fragments affixed to the solid support of the second screening array are selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46.

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67. A process for determining the presence or absence of aberrantly methylated DNA in cancer cells, said process comprising:

- 5           a.   preparing a first set of amplicons comprising (i)  
              contacting a nucleic acid sequence with an enzyme  
              which digests the nucleic acid sequences fragments  
              in which CpG islands are preserved to form a  
              digestion product comprising methylated and  
              unmethylated CpG island loci; (ii) attaching the  
10           fragments to linker primers to form linker primer  
              products; (iii) amplifying the linker primer  
              products to form amplicons; (iv) labeling the  
              amplicons;
- 15           b.   preparing a second set of amplicons comprising (i)  
              contacting nucleic acid sequences with an enzyme  
              which digests the nucleic acid sequences into  
              fragments in which CpG islands are preserved; (ii)  
              attaching the fragments to linker primers to form  
              linker primer products; (iii) contacting the linker  
20           primer product with a methylation-sensitive enzyme  
              which digests the linker primer products having  
              unmethylated CpG dinucleotide sequences but not  
              methylated CpG dinucleotide sequences to form a  
              second digestion product comprising methylated CpG  
25           island loci; (iv) amplifying the second digestion  
              product to form amplicons; (v) labeling the  
              amplicons;

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- 5 c. contacting the first set of amplicons with a first screening array comprising a plurality of nucleic acid fragments affixed to a solid support and determining the presence or absence of labeled amplicons bound to the plurality of nucleic acid fragments of the first screening array;
- 10 d. contacting the second set of amplicons with a second screening array which comprises a plurality of nucleic acid fragments affixed to a solid support wherein the plurality of nucleic acid fragments is identical to the plurality of nucleic acid fragments of the first screening array and determining the presence or absence of labeled amplicons bound to the plurality of nucleic acid fragments of the second screening array; and
- 15 e. observing whether the presence or absence of the first set of amplicons bound to the plurality of nucleic acid fragments of the first screening array is the same as the presence or absence of the second set of amplicons bound to the plurality of nucleic acid fragments of the second screening array.
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68. The process of claim 67 wherein the nucleic acid sequences are derived from a cancer tumor cell.

69. The process of claim 68 wherein the cancer tumor cell is a breast cancer cell, prostate cancer cell, colon cancer cell, lung cancer cell, liver cancer cell and ovarian cancer cell.
70. The process of claim 67 wherein the plurality of nucleic acid fragments affixed to the solid support of the first screening array and the plurality of nucleic acid fragments affixed to the solid support of the second screening array are derived from a CpG dinucleotide rich genomic library.
71. The process of claim 70 wherein the plurality of nucleic acid fragments affixed to the solid support of the first screening array and the plurality of nucleic acid fragments affixed to the solid support of the second screening array are CpG dinucleotide rich fragments which comprise a sequence of at least about 200 nucleotides of which at least about 50% are guanine and cytosine.
72. The process of claim 71 wherein at least 20 nucleic acid fragments are affixed to the solid support of the first screening array and the plurality of nucleic acid fragments of affixed to the solid support of the second screening array.

73. The process of claim 71 wherein the plurality of nucleic acid fragments affixed to the solid support of the first screening array and the plurality of nucleic acid fragments of affixed to the solid support of the second screening array each contain a promoter and a first exon of a gene.
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74. The process of claim 73 wherein at least 20 nucleic acid fragments are affixed to the solid support of the first screening array and the plurality of nucleic acid fragments of affixed to the solid support of the second screening array.
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75. The process of claim 74 wherein at least 100 nucleic acid fragments are affixed to the solid support of the first screening array and the plurality of nucleic acid fragments of affixed to the solid support of the second screening array.
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76. The process of claim 75 wherein at least 500 nucleic acid fragments are affixed to the solid support of the first screening array and the plurality of nucleic acid fragments of affixed to the solid support of the second screening array.
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77. The process of claim 67 wherein the solid support of the first screening array and the solid support of the second screening array comprises nylon, glass or silicon.

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78. The process of claim 67 wherein the enzyme is selected from the group comprising MseI, Tsp509I, NlaIII and BfaI and the methylation sensitive enzyme is selected from the group comprising BstU I, SmaI, SacII, EagI, MspI, HpaII, HhaI and BssHII.

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79. The process of claim 78 wherein the enzyme is MseI and the methylation sensitive enzyme is BstU I.

80. The process of claim 67 wherein the plurality of nucleic acid fragments affixed to the solid support of the first screening array and the plurality of nucleic acid fragments affixed to the solid support of the second screening array are selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43, SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46.

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